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**ABSTRACT**

Comparisons of the volatile leaf oil components for four populations of *J. excelsa* are reported. The major components are cedrol (25.4 - 29.3%),  $\alpha$ -pinene (21.6 to 41.8%), limonene (0.5 - 13.2%),  $\beta$ -phellandrene (0.4 - 9.2%) and (2E,4E)-decadienal (2.3 - 3.9%). The trees from Greece and Bulgaria were higher in limonene (13.2, 11.3%) and  $\beta$ -phellandrene (13.2, 11.3%) than the samples from Turkey and Cyprus. In contrast, trees from Turkey and Cyprus were higher in  $\alpha$ -pinene (41.7, 32.6%) than trees in Greece and Bulgaria (21.6, 24.3%). Fifteen compounds, normally found only in heartwood, were present in the leaf oil, with cedrol being the major component. Cedrol ranged, rather continuously, from 11.3 to 35.8%, with no chemical polymorphisms detected among 12 trees from 3 regions. Published online: [www.phytologia.org](http://www.phytologia.org) *Phytologia* 95(4): 279-285 (Nov. 1, 2013). ISSN 030319430

**KEY WORDS:** *Juniperus excelsa*, geographic variation, leaf oils, terpenes, cedrol,  $\alpha$ -pinene, limonene.

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*Juniperus excelsa* M.-Bieb. is a wide-ranging species (Fig. 1) from Greece to Turkey and perhaps far east as Azerbaijan. Farjon (2005, 2010) treated *J. polycarpos*, *J. p. var. seravschanica* and *J. p. var. turcomanica* as *J. excelsa* subsp. *polycarpos*. However, Adams and Schwarzbach (2012) and Adams (2013), utilizing DNA sequence data, recognized *J. excelsa* as well as *J. polycarpos*, *J. p. var. turcomanica* and *J. seravschanica*. Adams and Hojjati (2012), using sequences from 4 gene regions, failed to find *J. excelsa* in Iran, but did find *J. polycarpos*, *J. p. var. turcomanica* and *J. seravschanica* in Iran. Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had 0 or only 1 SNP difference compared with *J. polycarpos* var. *polycarpos* from Armenia (Adams and Hojjati, 2012).

The early papers on volatile leaf oils have been reported upon, and literature reviewed by Adams (1990a). More recently, Ucar and Balaban (2002) examined the volatile oil from sapwood (outside white wood next to the interior heartwood) from *J. excelsa* and reported 22.5% widdrol and 9% cedrol. The volatile oil from berries (seed cones) of *J. excelsa* is generally similar to leaf oil and Unlu et al. (2008) found berry oil from Turkey to contain 55.5%  $\alpha$ -pinene, 7.75% cedrol, 3.55% sabinene along with 51 other compounds.

The purpose of this paper is to present an updated analyses of the oils and geographic variation of *J. excelsa*.

## MATERIALS AND METHODS

**Plant material** - *J. excelsa*: Eskisehir, Turkey, Adams 13193 (9433-9435), Bulgaria, Adams 14056 (13720-13724), Alex Tashev, 2012-1-JE -5-JE, 42° 01' 22.0" N; 24° 28' 03.1" E, 356 m, Central Rhodopes, above the town of Kritchim, Reserve "Izgorialoto Gune", Lemos, Greece, Adams 6031 (5983-5985, 5987), Cyprus, Adams 13487, Bouchra Douaihy ns., bulk 5 trees. *J. procera*: Adams 6184-6188, near Addis Alem, Ethiopia, 40 km west of Addis Ababa on road to Guder, 2400 m, ca. 9° 02' N, 38° 23' E. Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

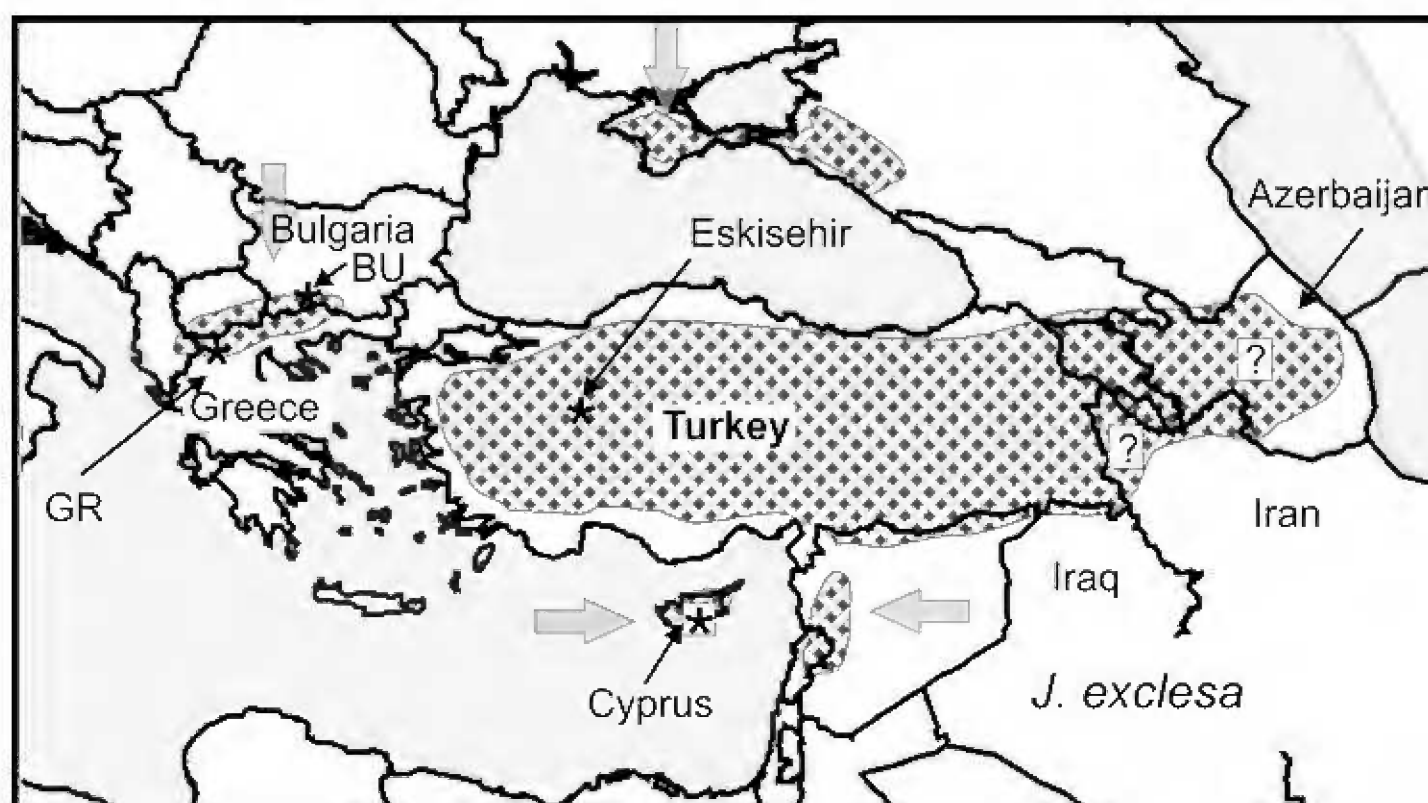


Figure 1. Distribution of *J. excelsa* from Adams (2011) with populations sampled in this study. Question marks (?) indicate questionable occurrences of *J. excelsa* in Azerbaijan and Iran.

Fresh or air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

## RESULTS AND DISCUSSION

Comparisons of the leaf components for the four populations are given in Table 1. The major components of the oils are cedrol (25.4 - 29.3%),  $\alpha$ -pinene (21.6 to 41.8%), limonene (0.5 - 13.2%),  $\beta$ -phellandrene (0.4 - 9.2%) and (2E,4E)-decadienal (2.3 - 3.9%). The trees from Greece and Bulgaria are higher in limonene (13.2, 11.3%) and  $\beta$ -phellandrene (13.2, 11.3%) than the samples from Turkey and Cyprus (Table 1). In contrast, trees from Turkey and Cyprus were higher in  $\alpha$ -pinene (41.7, 32.6%) than trees in Greece and Bulgaria (21.6, 24.3%).

In general, most *Juniperus* species produce two kinds of essential oils: leaf oils and heartwood oils and these oils have few components in common (Adams, 1991). *Juniperus excelsa*, along with *J. foetidissima*, *J. polycarpos* *J. p.* var. *turcomanica* and *J. seravschanica* have leaf oils that contain significant amounts of the heartwood oil components (cf.  $\alpha$ -cedrene  $\beta$ -cedrene, thujopsene, cuparene, cedrol, widdrol, etc., see Adams, 2011). For example, Adams (1990b) reported 4.4, 0.2, trace and 8.3% cedrol in the leaf oils from four trees of *J. foetidissima* from Greece. Whereas, Tunalier et al. (2004) reported 13.0 and 12.2% of cedrol and widdrol in the stem heartwood of *J. foetidissima* from Turkey. Ucar and Balaban (2002) analyzed the sapwood (white wood) of *J. excelsa*, Turkey, and reported the oil to contain 22.5% widdrol and 9.0% cedrol (these components are difficult to separate on non-polar columns and the mass spectra are nearly identical, so identification is often problematic). In the present case, *J. excelsa* leaf oils contain 15 compounds normally restricted to heartwood (boldface, Table 1). The trend from Greece-Bulgaria to Turkey-Cyprus is not seen in the heartwood components, as they are mostly uniform across the samples in this study.

When *Juniperus* species do contain heartwood components in the leaf oils, it is common to find chemical polymorphisms in cedrol between trees (see discussion of *J. foetidissima* above with 4.4, 0.2, trace and 8.3% of cedrol in leaf oils). However, in this study, the concentration of cedrol in the leaf oils is fairly continuous from 11.3% to 35.8% among the 12 individual trees examined (Table 2). The trees in the Bulgaria population were very uniform, ranging from 21.0 to 28.2% cedrol (Table 2).

The leaf oil of *J. procera* is included in table 1 for a comparison. It differs from *J. excelsa* oil in many components including  $\delta$ -3-carene, terpinolene, linalool, (E)-caryophyllene,  $\alpha$ -humulene,  $\gamma$ -eudesmol, 8- $\gamma$ -acetoxyelemol, and the presence of 10 diterpenes not found in *J. excelsa*. In addition, its leaf oil is, as found in most junipers, without the heartwood components (Table 1).

#### ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf essential oils from populations of *J. excelsa*. Bulgaria: Adams 14056 (13720-13724); Lemos, Greece: Adams 6031 (5983-5985, 5987), Eskisehir, Turkey; Adams 13193 (9433-9435) and Cyprus, Adams 13487 (bulk, 5 trees). Components that are typical of heartwood oil are highlighted in boldface. The oil of *J. procera* is from Addis Alem, Ethiopia, Adams 6184-6188.

KI	Compound	Bulgaria	Greece	Eskisehir	Cyprus	<i>procera</i>
921	tricyclene	t	0.1	0.2	0.1	t
932	$\alpha$ -pinene	24.3	21.6	41.7	41.8	32.6
945	$\alpha$ -fenchene	0.1	0.1	0.3	t	1.1
946	camphene	0.2	0.3	0.2	0.1	0.2
953	thuja-2,4(10)-diene	t	t	0.1	t	t
961	verbenene	t	t	t	t	t
969	sabinene	t	0.2	0.1	0.1	t
974	$\beta$ -pinene	0.5	0.5	0.7	0.4	3.1
988	myrcene	1.4	1.2	1.2	0.5	3.1
1002	$\alpha$ -phellandrene	t	t	0.1	t	t
1008	$\delta$ -3-carene	0.8	1.7	5.3	t	28.6
1014	$\alpha$ -terpinene	t	t	0.1	t	t
1020	p-cymene	0.5	0.4	0.6	0.1	0.2
1024	limonene	11.3	13.2	1.2	0.5	0.5
1025	$\beta$ -phellandrene	7.5	9.2	0.9	0.4	1.4
1044	(E)- $\beta$ -ocimene	t	t	t	t	t
1054	$\gamma$ -terpinene	0.5	0.4	0.5	0.3	t
1065	cis-sabinene hydrate	t	t	t	t	-
1086	terpinolene	0.8	0.7	1.1	0.3	3.2
1095	linalool	-	-	-	-	0.6
1097	trans-sabinene hydrate	t	t	t	t	-
1112	3-me-3-butenyl-methyl butanoate	0.4	0.1	t	t	-
1112	endo-fenchol	0.2	0.2	t	t	-
1118	cis-p-menth-2-en-1-ol	-	-	0.1	t	t
1122	$\alpha$ -campholenal	0.2	0.1	0.5	0.1	t
1132	cis-limonene oxide	-	-	-	-	0.2
1135	trans-pinocarveol	0.4	0.2	0.8	0.1	t
1137	trans-verbenol	-	-	0.2	-	0.1
1141	camphor	0.7	0.5	1.2	0.2	t
1145	camphene hydrate	t	t	0.1	t	-
1165	borneol	-	-	-	-	0.1
1166	p-mentha-1,5-dien-8-ol	t	t	-	t	t
1172	cis-pinocamphone	t	t	0.2	t	t
1174	terpinen-4-ol	t	0.2	0.1	0.1	0.2
1178	naphthalene	t	t	0.1	t	t
1179	p-cymen-8-ol	t	t	0.1	t	t
1186	$\alpha$ -terpineol	t	t	t	t	0.4
1204	verbenone	t	t	0.2	t	-
1215	trans-carveol	0.2	0.1	0.2	t	-
1218	endo-fenchyl acetate	t	0.2	0.1	t	-
1249	piperitone	t	t	0.1	t	-

KI	Compound	Bulgaria	Greece	Eskisehir	Cyprus	<i>procera</i>
1260	3-me-3-butenol hexanoate	0.2	0.1	-	-	-
1274	pregeijerene B	-	-	-	-	0.6
1284	bornyl acetate	t	0.6	0.4	0.1	0.2
1292	(2E,4Z)-decadienal	t	0.3	0.1	t	-
1319	(2E,4E)-decadienal	3.9	3.6	2.4	2.3	0
1387	$\beta$ -bourbonene	t	0.1	0.1	t	t
1389	$\beta$ -elemene	-	-	-	-	t
1390	7-epi-sesquithujene	0.2	0.1	0.1	0.3	-
<b>1410</b>	<b><math>\alpha</math>-cedrene</b>	<b>1.0</b>	<b>1.1</b>	<b>0.8</b>	<b>1.7</b>	-
<b>1413</b>	<b><math>\beta</math>-funebreene</b>	<b>1.0</b>	<b>0.9</b>	<b>0.7</b>	<b>1.8</b>	-
1417	(E)-caryophyllene	-	-	-	-	0.8
<b>1419</b>	<b><math>\beta</math>-cedrene</b>	<b>1.1</b>	<b>1.0</b>	<b>0.5</b>	<b>1.0</b>	-
<b>1429</b>	<b>cis-thujopsene</b>	<b>0.3</b>	<b>0.4</b>	<b>0.3</b>	<b>0.8</b>	-
1451	trans-muurolo-3,5-diene	0.2	0.1	0.1	0.7	-
1452	$\alpha$ -humulene	0.1	0.2	0.1	0.2	3.8
1454	(E)- $\beta$ -farnesene	0.1	0.2	0.2	0.3	-
<b>1469</b>	<b><math>\beta</math>-acoradiene</b>	<b>0.1</b>	<b>0.1</b>	<b>0.2</b>	<b>0.4</b>	-
1475	trans-cadina-1(6),4-diene	0.4	0.3	0.2	0.7	-
1480	germacrene D	0.8	0.8	0.6	1.2	2.2
1493	trans-muurolo-4(14),5-diene	0.6	0.4	0.2	1.5	-
1493	epi-cubebol	-	-	0.3	-	-
1496	valencene	0.6	0.5	0.3	t	-
<b>1500</b>	<b><math>\beta</math>-himachalene</b>	-	<b>t</b>	<b>0.1</b>	-	-
<b>1504</b>	<b>cuparene</b>	-	<b>t</b>	<b>0.1</b>	<b>t</b>	-
<b>1506</b>	<b>(Z)-<math>\alpha</math>-bisabolene</b>	-	-	<b>0.1</b>	<b>t</b>	-
<b>1512</b>	<b><math>\alpha</math>-alaskene</b>	<b>0.4</b>	<b>0.3</b>	<b>0.2</b>	<b>0.2</b>	-
1513	$\gamma$ -cadinene	-	-	-	-	t
1514	cubebol	0.8	0.7	0.4	1.2	-
1521	trans-calamenene	0.5	0.2	0.2	0.5	-
1522	$\delta$ -cadinene	0.5	0.3	0.3	0.6	0.3
<b>1532</b>	<b><math>\gamma</math>-cuparene</b>	<b>0.3</b>	<b>0.2</b>	<b>0.2</b>	<b>0.5</b>	-
1574	germacrene D-4-ol	-	-	-	-	0.1
<b>1589</b>	<b>allo-cedrol</b>	<b>1.7</b>	<b>2.0</b>	<b>1.9</b>	<b>2.4</b>	-
<b>1600</b>	<b>cedrol</b>	<b>25.5</b>	<b>29.3</b>	<b>25.4</b>	<b>27.5</b>	-
1608	humulene epoxide II	t	t	t	t	0.3
1608	$\beta$ -oplophenone	t	t	t	t	-
1627	1-epi-cubenol	0.8	0.7	0.5	0.7	-
1630	$\gamma$ -eudesmol	-	-	-	-	0.6
<b>1632</b>	<b><math>\beta</math>-acorenol</b>	<b>t</b>	<b>0.1</b>	<b>0.1</b>	<b>0.2</b>	-
1638	epi- $\alpha$ -cadinol	t	t	t	t	-
1640	epi- $\alpha$ -muurolol	t	t	t	t	-
1645	cubenol	t	t	0.1	t	-
1649	$\beta$ -eudesmol	-	-	-	-	1.1
1652	$\alpha$ -eudesmol	-	-	-	-	1.6
1653	$\alpha$ -cadinol	t	t	t	t	-
1661	sesquiterpene <u>85,57,41,238</u>	1.1	0.9	1.0	1.2	-
<b>1668</b>	<b><math>\beta</math>-atlantone</b>	<b>0.7</b>	<b>0.5</b>	<b>0.6</b>	<b>0.7</b>	-
1670	bulnesol	-	-	-	-	0.3

KI	Compound	Bulgaria	Greece	Eskisehir	Cyprus	<i>procera</i>
1685	germacra-4(15),5,10-trien-1-al	-	-	-	-	0.1
<b>1713</b>	<b>cedroxyde</b>	-	-	<b>0.1</b>	<b>t</b>	-
1792	8- $\alpha$ -acetoxyelemol	-	-	-	-	0.9
1958	iso-pimara-8(14),15-diene	-	-	-	-	t
1987	manoyl oxide	t	0.2	0.1	t	3.3
2055	abietatriene	t	0.4	t	t	0.2
2087	abietadiene	-	-	-	-	1.9
2105	iso-abienol	-	-	-	-	0.8
2153	abieta-8(14),13(15)-diene	-	-	-	-	t
2181	sandaracopimarinal	-	-	-	-	0.1
2269	sandaracopimarinol	-	-	-	-	t
2282	sempervirol	-	-	-	-	0.2
2298	4-epi-abietal	0.2	0.2	0.2	t	0.8
2314	trans-totarol	-	-	-	-	2.4
2331	trans-ferruginol	-	-	-	-	0.5
2401	abietol	-	-	-	-	0.1

Table 2. Variation in cedrol (% total oil) among individual trees in populations of *J. excelsa*. Note that the Cyprus value for cedrol is from bulked leaves from 5 trees.

Bulgaria	22.1	24.4	26.0	28.2	24.4
Greece	23.0	18.8	28.0	35.8	
Eskisehir	27.6	11.3	22.1		
Cyprus	27.5				